A series of vectors to construct \textit{lacZ} fusions for the study of gene expression in \textit{Schizosaccharomyces pombe}

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Abstract We have constructed a series of plasmids to facilitate the fusion of promoters with or without coding regions of genes of \textit{Schizosaccharomyces pombe} to the \textit{lacZ} gene of \textit{Escherichia coli}. These vectors carry a multiple cloning region in which fission yeast DNA may be inserted in three different reading frames with respect to the coding region of \textit{lacZ}. The plasmids were constructed with the \textit{ura4}$^+$ or the \textit{his3}$^+$ marker of \textit{S. pombe}. Functionality of the plasmids was tested measuring in parallel the expression of fructose 1,6-bisphosphatase and \textit{β}-galactosidase under the control of the \textit{fbp1}$^+$ promoter in different conditions.

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Key words: Expression vector; \textit{lacZ} fusion; \textit{Schizosaccharomyces pombe}

1. Introduction

Analysis of factors that influence gene expression is one important question in different fields of current biology. Ideally, quantification of changes in gene expression between different situations needs careful analysis of specific mRNAs. Since the available techniques to perform these measurements are laborious they are not practical when a large number of samples needs to be processed as happens during the characterization by deletion analysis of regulatory regions in the promoter of a gene. An alternative method that is widely used is the fusion of promoter regions of the gene under study to the coding sequence of a reporter gene. The changes in the amount or activity of the reporter protein are accepted as an estimate of those in the expression of the gene itself. Although this method is indirect and several factors different from gene expression may influence the amount of protein measured, it has become very popular due to its simplicity and versatility. Among the several reporter genes available (see for example [1]) the most commonly used is still the \textit{lacZ} gene from \textit{Escherichia coli} that encodes \textit{β}-galactosidase.

To facilitate the construction of \textit{lacZ} fusions in \textit{Saccharomyces cerevisiae}, Myers et al. [2] designed plasmids in which the sites of the multiple cloning region of pUC8 and pUC18 could be used to insert yeast DNA fragments in three different reading frames with respect to the coding region of \textit{lacZ}. Due to the increasing interest in the study of regulation of gene reading frames with respect to the coding region of \textit{lacZ}. The plasmids were constructed with the \textit{ura4}$^+$ or the \textit{his3}$^+$ marker of \textit{S. pombe}. We evaluated the functionality of these vectors by assaying in different growth conditions the expression of the \textit{lacZ} gene under the control of the promoter of the \textit{fbp1}$^+$ gene [5]. The vectors presented can, in principle, be used to study the regulation of other genes of the fission yeast. (The collection of vectors constructed has been deposited in the Spanish Type Culture Collection$^2$.)

2. Materials and methods

2.1. Yeast and bacterial strains and culture conditions

\textit{S. pombe} strains CJM094 $^{h-}$ \textit{ade6-204 ura4-294} (originally provided by S. Moreno, Salamanca, Spain) and TPY59 $^{h-}$ \textit{ade6-M210 ura4-D18 leu1-32 his3-D2} (provided by K.L. Gould, Nashville, TN, USA) were used throughout this work. They were grown at 30$^\circ$C in a synthetic medium [6] with 2% glucose or 3% glycerol+0.05% glucose as carbon sources and the adequate auxotrophic requirements at a final concentration of 100 µg/ml. Transformation of \textit{S. pombe} was done with lithium chloride as in [6].

\textit{Escherichia coli} DH5$^\alpha$ was used for plasmid multiplication. It was grown on LB [7] supplemented with 50 µg/ml ampicillin.

2.2. Plasmids and DNA manipulations

The following plasmids were used: pUC18 [8], p Bluescript$^\alpha$KS$^+$ (Stratagene), YEp353-358 and YEp356R-358R [2], obtained from the ATCC (Rockville, MD, USA), pREP4 [9], pAF1[10] (sent by K.L. Gould, Nashville, TN, USA) and pAOV6 [5] (sent by M. Boutry, Louvain la Neuve, Belgium).

2.3. Construction of promoter fusions

The plasmids that contain the \textit{fbp1}$^+$ promoter fused in frame with the \textit{lacZ} coding sequence were constructed as follows. The promoter region of \textit{fbp1}$^+$ was amplified by PCR using DNA from plasmid pAV06 using the following oligonucleotides as primers: upstream 5'-CGTTGCT-3' and downstream 5'-GCATCC-3'. The 1.1-kbp fragment was ligated respectively into plasmids pSPI-353 and pSPE-353 (see Section 3), previously cut with EcoRI and BamHI. The cloned fragment contains 3 amino acids from the coding region of \textit{S. pombe} fructose 1,6-bisphosphatase.

2.4. Extracts and enzymatic assays

Yeast extracts were prepared by shaking with glass beads in 20 mM imidazole, pH 7 as described [11]. For \textit{β}-galactosidase assay the centrifugation step was omitted. \textit{β}-Galactosidase was assayed and units defined as in [12] with a centrifugation step before reading optical density. Fructose 1,6-bisphosphatase was assayed as in [13]. Protein was determined by the method of Lowry et al. [14] using the Pierce Protein Assay Reagent.

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3. Results and discussion

3.1. Construction of episomal and integrative lacZ fusion plasmids for expression of β-galactosidase in the fission yeast carrying the ura4⁺ or his3⁺ markers

Vectors to allow fusion of DNA fragments in three different reading frames to the E. coli lacZ gene were first constructed by Minton [15]. Myers et al. [2] made use of these vectors to construct a series of S. cerevisiae-E. coli shuttle plasmids. We have modified these last vectors to introduce in them the genes ura4⁺ or his3⁺ and the ARS201 fragment from S. pombe. Plasmids YEp353-358 and YEp356R-358R were digested with NsiI (AvaIII) and the 1.6-kbp band containing the S. cerevisiae URA3 gene and a part of the 2 μ sequence was eliminated. We introduced in these treated plasmids different cassettes with ura4⁺, ura4⁺-ARS201 or his3⁺-ARS201 as PstI fragments (see below).

3.2. Preparation of the S. pombe cassettes ura4⁺, ura4⁺-ARS-201 and his3⁺-ARS-201 for constructions

The ura4⁺ gene was prepared to be inserted in the NsiI-digested plasmids as follows. The 1.85-kbp HindIII-HindIII fragment from pREP4 carrying the S. pombe ura4⁺ gene was introduced into pBluescript®KS⁺ digested with HindIII;

Fig. 1. Basic structure of the plasmids to construct lacZ fusions for expression in S. pombe. Plasmids with the ura4⁺ marker are available as episomal or integrative while those with the his3⁺ marker exist only as episomal. Sites in bold are unique in the corresponding plasmid. Plasmids pSPI356R to 358R, pSPE356R to 358R and pSPE376R to 378R have the polylinker in the opposite orientation. For details of the constructions see text.
the resulting plasmid was digested with SalI and BamHI and the 1.85-kbp fragment was cloned into the SalI and BamHI digested plasmid pUC18 previously cut with SmaI and EcoRI, blunt ended and religated. The restriction sites SalI and ClaI flank ura4‡ were eliminated by cutting and filling-in to yield plasmid pMJ1. The EcoRI site also flanking ura4‡ was eliminated from this plasmid by cutting and filling-in to produce plasmid pMJ2.

The ARS201 fragment was obtained as a 1.2-kbp EcoRI-EcoRI fragment from pREP4. It was blunt ended and cloned into pMJ1 digested with EcoRI and blunt ended producing plasmid pMJ3.

The his3‡-ARS-201 cassette was prepared to be inserted in the SfiI digested plasmids as follows: The 1.9-kbp SmaI-SalI blunt ended fragment from pAF1 with the gene his3‡, was introduced into a 3.9-kbp fragment of pMJ3 obtained after blunt ending the product of a HindIII digestion to yield plasmid pMJ4.

### 3.3. Construction of a set of integrative plasmids

For the construction of pSPI plasmids (Schizosaccharomyces pombe Episomal) the 3-kbp PstI-PstI fragment from plasmid pMJ3, carrying the ura4‡-ARS201 cassette was cloned into all plasmids digested with SfiI as above yielding plasmids pSPI353 to pSPI358 and pSPI356R to pSPI358R (Fig. 1). Plasmids pSPI353 to pSPI355 carry the multiple cloning region of pUC8 while the rest carry that of pUC18 [16]. To allow an easy reference to the corresponding plasmids of S. cerevisiae and avoid proliferation of designations, the ura4‡ containing plasmids carry the same numbers as those of Myers et al. [2] with the letters SP to indicate S. pombe.

To increase the versatility of these plasmids and allow their use in strains with a marker different from ura4‡ we generated another series of episomal plasmids carrying the his3‡ marker.

### 3.4. Construction of a set of episomal plasmids

For the construction of the pSPE plasmids (Schizosaccharomyces pombe Episomal) the 3-kbp PstI-PstI fragment from plasmid pMJ3, carrying the ara 4‡-ARS201 cassette was cloned into all plasmids digested with XhoI and EcoRI, blunt ended and religated. The restriction sites SfiI and XhoI flanking ura4‡ were eliminated by cutting and filling-in to yield plasmid pMJ1. The EcoRI site also flanking ura4‡ was eliminated from this plasmid by cutting and filling-in to produce plasmid pMJ2.

The FRPase and β-galactosidase (β-Gal) activities (mU/mg protein) of S. pombe strains transformed with episomal (pSPI353-fbp1) or integrative (pSPI353-fbp1) plasmids carrying fbp1lacZ fusions

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Reading frame and restriction sites</th>
<th>Small</th>
<th>SalI</th>
<th>HindIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>pSPI353/pSPE353/pSPE373</td>
<td>gaa ttc gcg ggg gat ccc tgc acc tgc acc cca gct tgc gat ccc</td>
<td>EcoRI</td>
<td>Small</td>
<td>BamHI</td>
</tr>
<tr>
<td>pSPI354/pSPE354/pSPE374</td>
<td>gaa ttc gcg ggg gat ccc tgc acc tgc acc cca gct tgc gat ccc</td>
<td>EcoRI</td>
<td>Small</td>
<td>BamHI</td>
</tr>
<tr>
<td>pSPI355/pSPE355/pSPE375</td>
<td>g aat tcc gcg gga tcc gcc agc cct gcc gca gca gca acc tgc gat ccc</td>
<td>EcoRI</td>
<td>Small</td>
<td>BamHI</td>
</tr>
<tr>
<td>pSPI356/pSPE356/pSPE376</td>
<td>g aat tcc gcg gga tcc gcc agc cct gcc gca gca gca acc tgc gat ccc</td>
<td>EcoRI</td>
<td>Small</td>
<td>BamHI</td>
</tr>
<tr>
<td>pSPI357/pSPE357/pSPE377</td>
<td>g aat tcc gcg gga tcc gcc agc cct gcc gca gca gca acc tgc gat ccc</td>
<td>EcoRI</td>
<td>Small</td>
<td>BamHI</td>
</tr>
<tr>
<td>pSPI358/pSPE358/pSPE378</td>
<td>g aat tcc gcg gga tcc gcc agc cct gcc gca gca gca acc tgc gat ccc</td>
<td>EcoRI</td>
<td>Small</td>
<td>BamHI</td>
</tr>
<tr>
<td>pSPI356R/pSPE356R/pSPE376R</td>
<td>g aat tcc gcg gga tcc gcc agc cct gcc gca gca gca acc tgc gat ccc</td>
<td>EcoRI</td>
<td>Small</td>
<td>BamHI</td>
</tr>
</tbody>
</table>

### Table 2

Fructose 1,6-bisphosphatase (FbPase) and β-galactosidase (β-Gal) activities (mU/mg protein) of S. pombe strains transformed with episomal (pSPI353-fbp1) or integrative (pSPI353-fbp1) plasmids carrying fbp1lacZ fusions

<table>
<thead>
<tr>
<th></th>
<th>pSPI353-fbp1</th>
<th>pSPI353-fbp1</th>
</tr>
</thead>
<tbody>
<tr>
<td>FbPase</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>β-Gal</td>
<td>140</td>
<td>70</td>
</tr>
<tr>
<td>Glucose (X)</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>Glucose (S)</td>
<td>5500</td>
<td>2100</td>
</tr>
<tr>
<td>Glycerol (X)</td>
<td>90</td>
<td>80</td>
</tr>
</tbody>
</table>

The transformed yeasts were grown as described in Section 2 and harvested during the exponential (X) or the stationary (S) phase of growth. Enzymatic activities were assayed as described in Section 2.
mids digested with NsiI, yielding pSPE373 to pSPE378 and pSPE376R to pSPE378R (Fig. 1).

The structures of the multiple cloning sites of these vectors based on the sequences provided by Myers et al. [2] are shown in Table 1.

3.5. Expression of β-galactosidase from the vectors

To test the functionality of the plasmids constructed we measured the expression of β-galactosidase driven from the fbp1‡ promoter in different growth conditions using the episomal and integrative plasmids pSPE353 and pSPI353. This last construct was integrated in the chromosomal ura4‡ locus after linearization with StuI that is internal to the ura4‡ gene and is not present in the rest of the construct sequence. The vectors did not express β-galactosidase in S. pombe in the absence of sequences of a yeast promoter (results not shown). As may be seen in Table 2 the values of β-galactosidase expressed under the control of the fbp1‡ promoter varied in the same direction as those of the fructose 1,6-bisphosphatase measured in the same cultures. Parallel assays with plasmids carrying the his3‡ marker (results not shown) demonstrated the adequacy of the other constructs.

Constructs with the plasmids described may also be useful to localize gene products intracellularly as antibodies against β-galactosidase are commercially available.

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